

## Structure of QEA Fragments

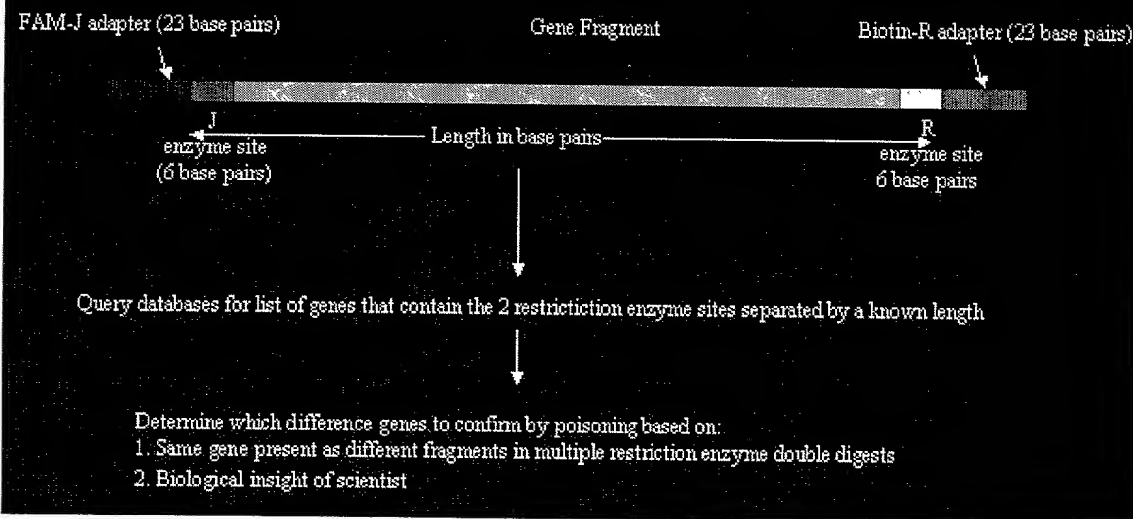


FIG. 1. Structure of QEA fragments. The FAM labeled J adapter (23 base pairs long) and biotin labeled R region (23 base pairs long) are oriented to the gene fragment as shown. The J enzyme site and R enzyme site are the restriction sites for their respective adapters (6 bp long). The larger central region plus the 2 restriction enzyme sites originate from a targeted nucleic acid sequence.

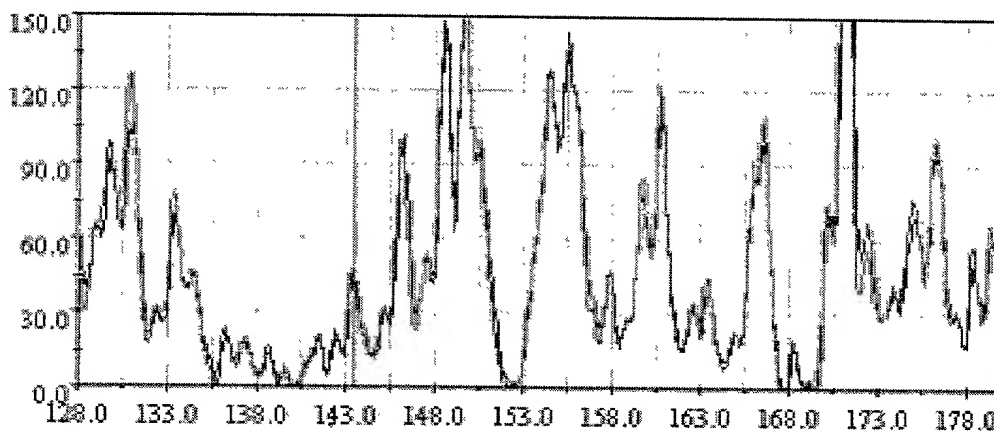


FIG. 2. Example of QEA peak traces from rat liver BglIII BspHI double digest. Traces show peaks in the 120-180 bp region.

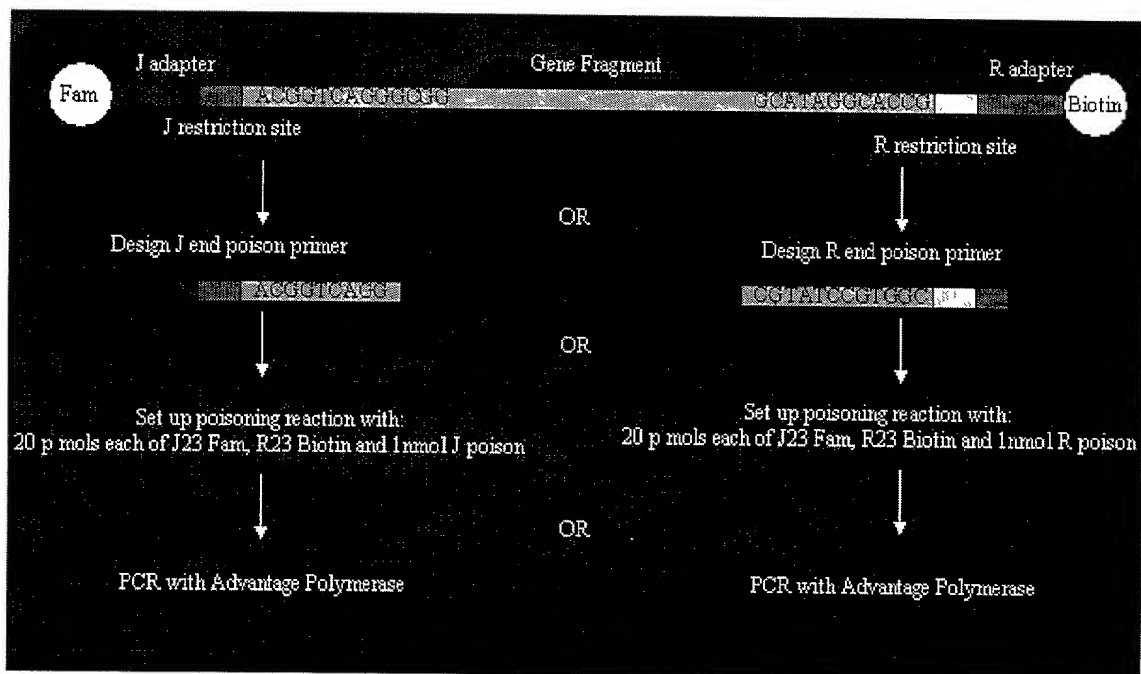


FIG. 3. Oligo-competition set up. Oligo-competition primers are designed on the J or R side based on the predicted sequence of the GeneCalled™ fragment. Oligo-competition reactions involve J23 and R23 primers with a fifty fold excess of the oligo-competition primers.

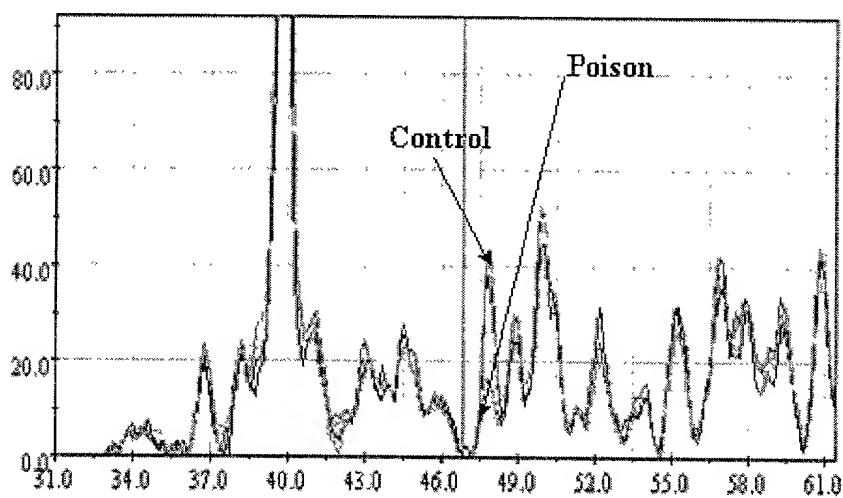


FIG. 4. Example of oligo-competition traces superimposed over control (no oligo-competition primer reactions) traces. (The traces are similar except where labeled. In this example the QEA peak at 48 bp was accurately sized, GeneCalled™, and poisoned.

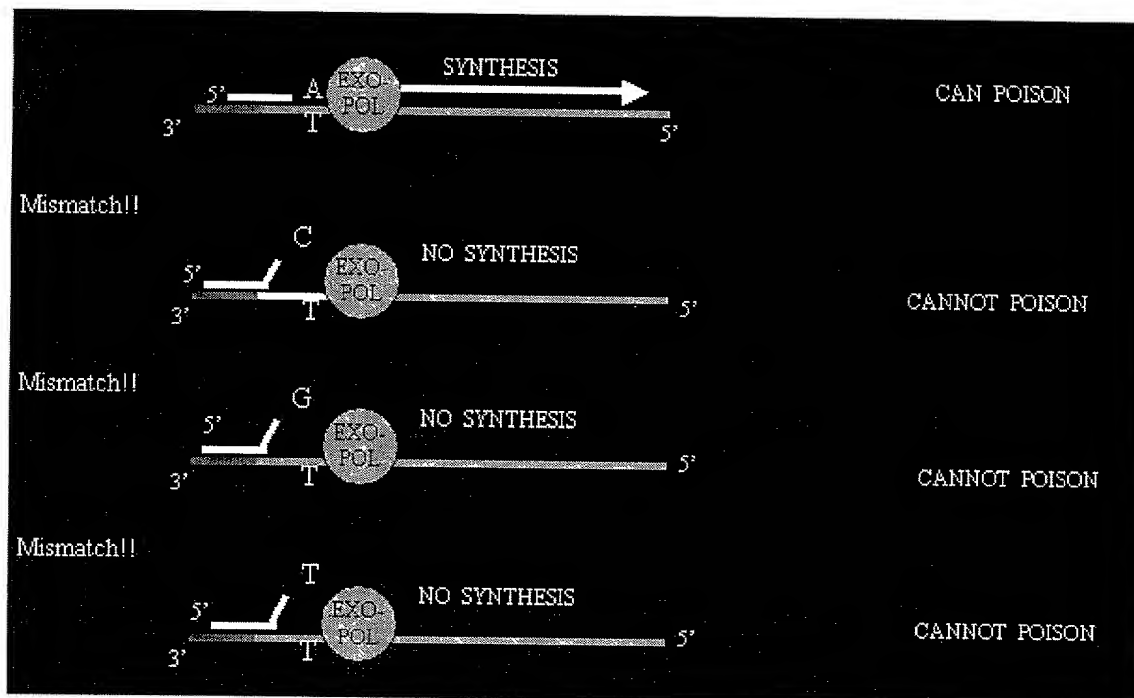
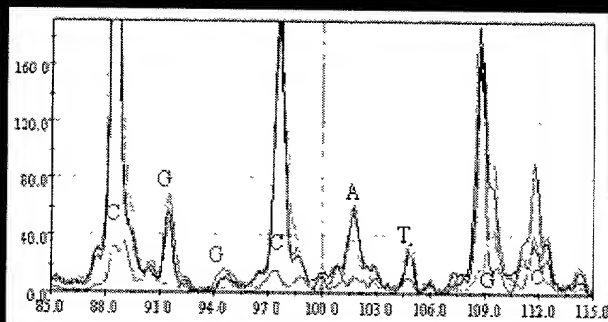


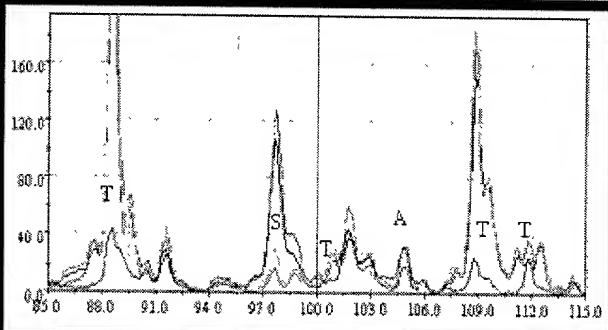
FIG. 5. Oligo-competition principle. Oligo-competition primers that have a perfect match at their 3' end with respect to the template strand are able to support DNA synthesis with an exonuclease-deficient DNA polymerase, and are therefore able to compete with J23 and R23 primers leading to the oligo-competition of these peaks. In contrast, QEA peaks with mismatches at the their 3' termini cannot support DNA synthesis, and will not be oligo-competitioned.

# Rat Liver i0m0

A POISON  
C POISON  
G POISON  
T POISON



R end poison (i0)

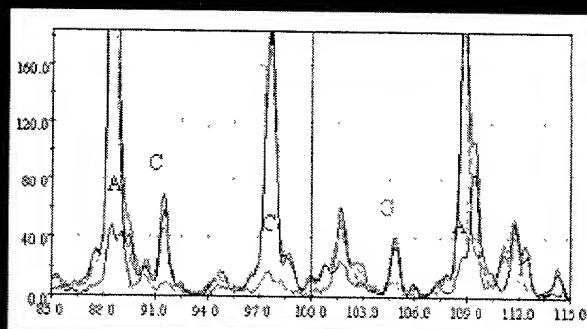


J end poison (m0)

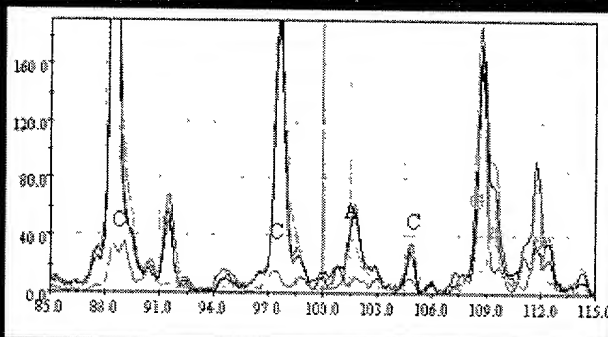
FIG. 6. Example of identification of the first base on the 3' side of the restriction enzyme sites on the R and J side for each QEA peak in the BspHI-BglII double digest of rat liver cDNA. (see text for details)

# Rat Liver i0m0

A POISON  
C POISON  
G POISON  
T POISON



R end poison (i0)  
2nd nucleotide



J end poison (m0)  
2nd nucleotide

FIG. 7. Phasing traces for identification of the 2<sup>nd</sup> nucleotide on the 3' side of the BglII (top panel) and BspHI (bottom panel) sites.

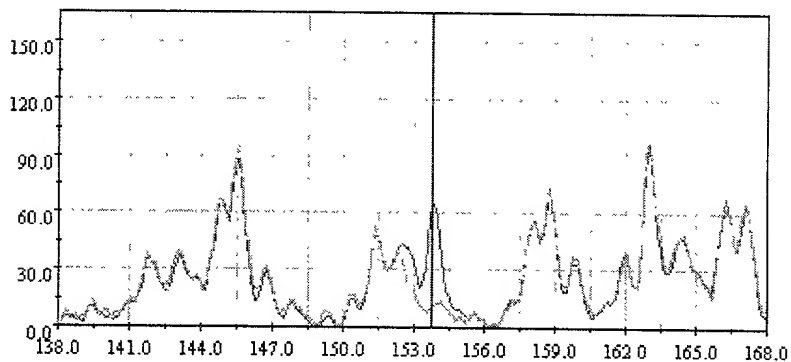


FIG. 8. Oligo-competition reaction (gray trace is the poison trace; black is the control trace) using an oligo-competition primer designed using the rat glycogen synthase gene confirms that GeneCall for the 153.8 bp BamHI-HindIII fragment.

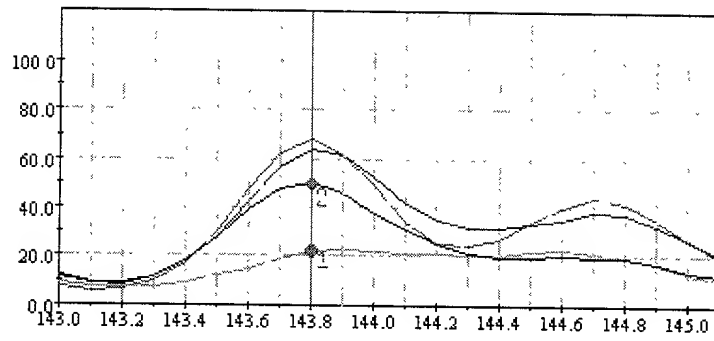


FIG. 9. Oligo-competition traces for a specific pair of cDNA cut sites, scaled and normalized intensity vs. fragment length, bp. One nucleotide is identified.

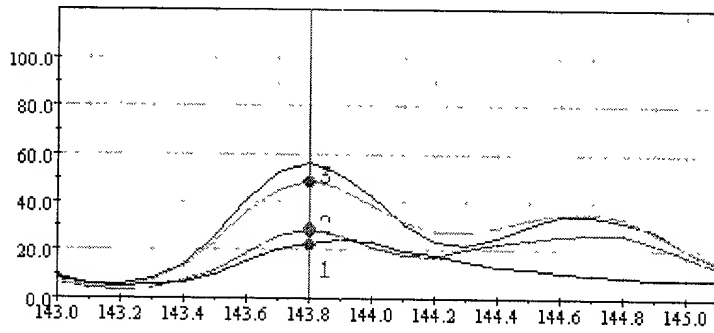


FIG. 10. Oligo-competition traces for a specific pair of cDNA cut sites, scaled and normalized intensity vs. fragment length, bp. Two nucleotides are identified.

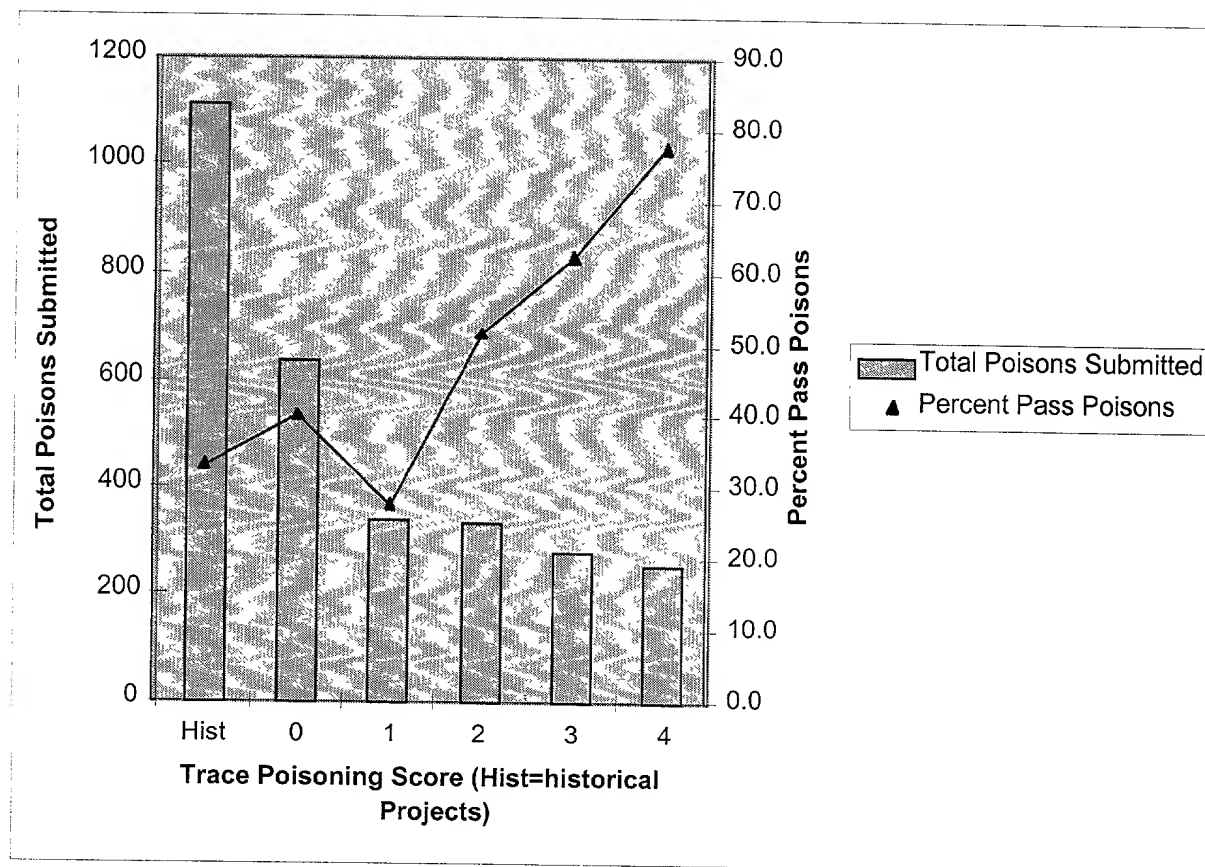


FIG. 11. Overall association of the trace oligo-competition score and trace oligo-competition effectiveness compared to historical data.

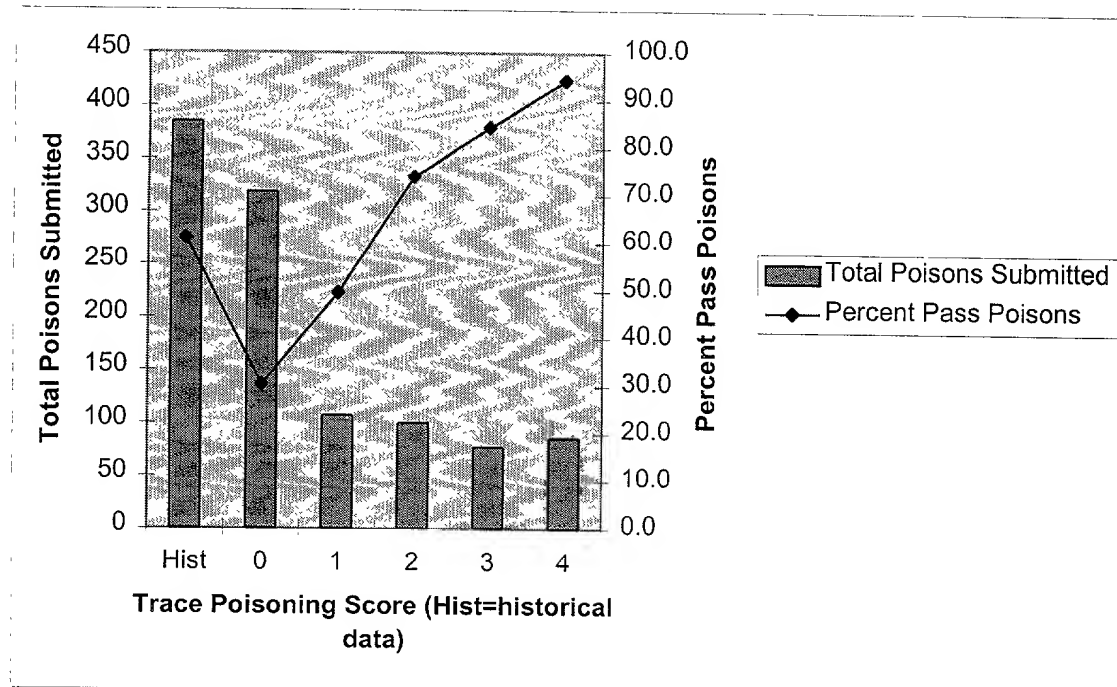


FIG. 12. Overall Association of the trace oligo-competition score and oligo-competition success among GeneCalls from Sized SeqCalling database.

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